AR201-13895A

The Flavor And Fragrance High Production Volume Consortia

The Aromatic Consortium

Test Plan For Phenethyl alcohol

Phenethyl alcohol **CAS No. 60-12-8**

FFHPVC Aromatic Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The Flavor and Fragrance High Production Volume Chemical Consortia
1620 I Street, NW, Suite 925
Washington, DC 20006
Phone: 202-331-2325
Fax: 202-463-8998

List of Member Companies

BASF

EASTMAN CHEMICAL COMPANY

FIRMENICH, INCORPORATED

GIVAUDAN CORPORATION

HAARMANN & REIMER CORPORATION

INTERNATIONAL FLAVOR & FRAGRANCES, INC.

KOSA

LYONDELL CHEMICAL COMPANY

POLAROME INTERNATIONAL

PROCTOR & GAMBLE

QUEST

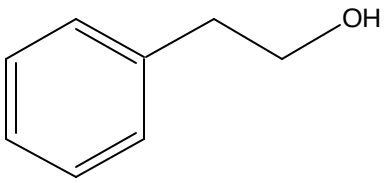
RHODIA INC.

SUMITOMO CHEMICAL COMPANY

Table of Contents

The HPV Challenge Test Plan for Phenethyl alcohol

1 IDENTITY OF SUBSTANCE



Phenethyl alcohol

$C_8H_{10}O$

CAS No. 60-12-8

Synonyms:

Benzeneethanol
Ethanol, 2-phenyl-
(2-Hydroxyethyl)benzene
PEA
beta-Phenethyl alcohol
2-Phenylethanol

2 CHEMICAL ANALYSIS

2.1 Introduction

In October of 1999, members of the United States flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Aromatic Consortium, as a member of the FFHPVC serves as an industry consortium to coordinate testing activities for aromatic substances under the Chemical Right-to-Know Program. Fourteen (14) companies are current members of the Aromatic Consortium. The Aromatic Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The test plan, chemical analysis and robust summaries presented below are the first phase of the Aromatic Consortium's commitment to the Chemical Right-to-Know Program.

2.2 Background Information

Phenethyl alcohol (PEA) or 2-phenylethanol is a simple aromatic primary alcohol. It is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavoring substance and is considered by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance [Hall, 1960]. In addition, a group of 42 phenethyl alcohol, phenylacetaldehyde, phenylacetic acid and related phenethyl esters and acetals have been approved for use as flavoring agents by both the FDA (CFR 172.515) and the World Health Organization's Joint Expert Committee on Food Additives [JECFA, 2002].

Phenethyl alcohol occurs naturally in more than 200 foods [Maarse *et al*., 2000]. Quantitative natural occurrence data indicate that oral intake of phenethyl alcohol occurs predominantly from consumption of foods such as beer, wine, whiskey, olive oil, grapes, green and black tea, apple juice and coffee [Stofberg and Grundschober, 1987]. It has been estimated that approximately 700,000 kg of phenethyl alcohol is consumed annually as a natural component of foods.

Phenethyl alcohol is the main component of rose oil and is also found in neroli oil, ylang-ylang oil, carnation oil, and geranium oils. Therefore, phenethyl alcohol is used as a fragrance ingredient because of its rose-like odor in a wide variety of consumer products ranging from hydroalcoholic (typically in 70% ethanol) type products such as colognes and *eaux de toilette*, to cosmetics, soaps and detergents [Opdyke, 1975]. Such uses consumed approximately 1,000,000 pounds (lbs)/year in 1975 [Opdyke, 1975].

Phenethyl alcohol is also used as a flavor ingredient with an annual volume of use reported to be 2500 kg/year in the USA and 9900 kg/year in Europe [Lucas *et al*., 1999; IOFI, 1995]. Therefore, greater than 99% of oral intake of phenethyl alcohol occurs from consumption of food containing naturally occurring phenethyl alcohol compared to the intake from its intentional use as a flavoring substance.

Phenethyl alcohol may be synthesized by a variety of methods including a Friedel-Crafts reaction of benzene and ethylene oxide, and by hydrogenation of styrene oxide [Bauer and Garbe, 1985].

2.3 Reactivity and Metabolism

When ingested in traditional foods or intentionally added as a flavor ingredient of food, phenethyl alcohol is rapidly absorbed from the gastrointestinal tract. Once absorbed, phenethyl alcohol is oxidized to yield phenylacetic acid that is subsequently conjugated and excreted in the urine [Williams, 1959; El Marsy *et al*., 1956; James *et al*., 1972; Caldwell, 1987; Sangster and Lindley, 1986; Hawkins and Mayo, 1986].

Phenethyl alcohol is readily oxidized to phenylacetaldehyde by an assortment of NAD^+-dependent alcohol and aldehyde dehydrogenases [Bosron and Li, 1980]. The highest

activity of mammalian alcohol dehydrogenases (ALDH) occurs in the liver where they exhibit broad substrate specificity for the oxidation of primary aliphatic and aromatic alcohols. Human liver ALDH shows decreased K_m[1] with increasing lipophilicity. However, V_{max}[2] remains essentially constant suggesting that the rate-limiting step does not involve the binding or release of the alcohol or aldehyde intermediate [Pietruszko *et al*., 1973].

Once formed, phenylacetaldehyde is oxidized by inducible aldehyde dehydrogenases from rat liver cytosol. In the rat, these isoenzymes can be induced by phenobarbital [Simpson *et al*., 1985]. The K_m and V_{max} values of human mitochondrial aldehyde dehydrogenase (ALDH-2) and cytosolic isoenzyme (ALDH-1) for oxidation of phenylacetaldehyde indicate rapid conversion to phenylacetic acid [Klyosov, 1996].

Phenylacetaldehyde, 3- and 4-chlorophenylacetaldehyde are effectively oxidized to the corresponding phenylacetic acid derivatives when incubated with rat hepatic microsomal dehydrogenase containing NAD^+ as a coenzyme. The rates of oxidation for the 3- and 4-chloro derivatives are markedly slower than that of the parent phenylacetaldehyde [Martini and Murray, 1996]. In dogs, 32% of a 1,900 mg/kg bw dose of phenylacetaldehyde (No. 1002) given to dogs is rapidly oxidized and excreted as the glycine conjugate within 48 hours [Kay and Raper, 1922].

Phenylacetic acid is a normal component of human urine (250-500 mg/24 hours) and human blood (500 ng/ml) [Sandler *et al*., 1982], forming mainly from the breakdown of phenylalanine by intestinal bacteria [Seakins, 1971] or *via* oxidative deamination of endogenous phenethylamine [Seakins, 1971; Richter, 1938]. The following studies demonstrate that the metabolism and excretion of phenethyl alcohol occur *via* a pathway used by humans and other animals to metabolize endogenous substances. When administered orally, phenethylamine is rapidly metabolized to phenylacetylglutamine. Two human subjects, each fed a 300 mg dose of S-phenethylamine, excreted 60-62% of the administered dose as conjugated phenylacetic acid in the urine within 2 - 4.5 hours

[1] The Michaelis-Menten constant, K_m, is the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity. Michaelis-Menten equation: $v_0 = V_{max}[S]/K_m+[S]$ where v_0=initial rate at substrate concentration [S].

[Seakins, 1971; Richter, 1938]. Also, greater than 80% of [^{14}C]-S-phenethylamine fed to mice was rapidly excreted from urine as the glutamine conjugate of [^{14}C]-phenylacetic acid [Block, 1953].

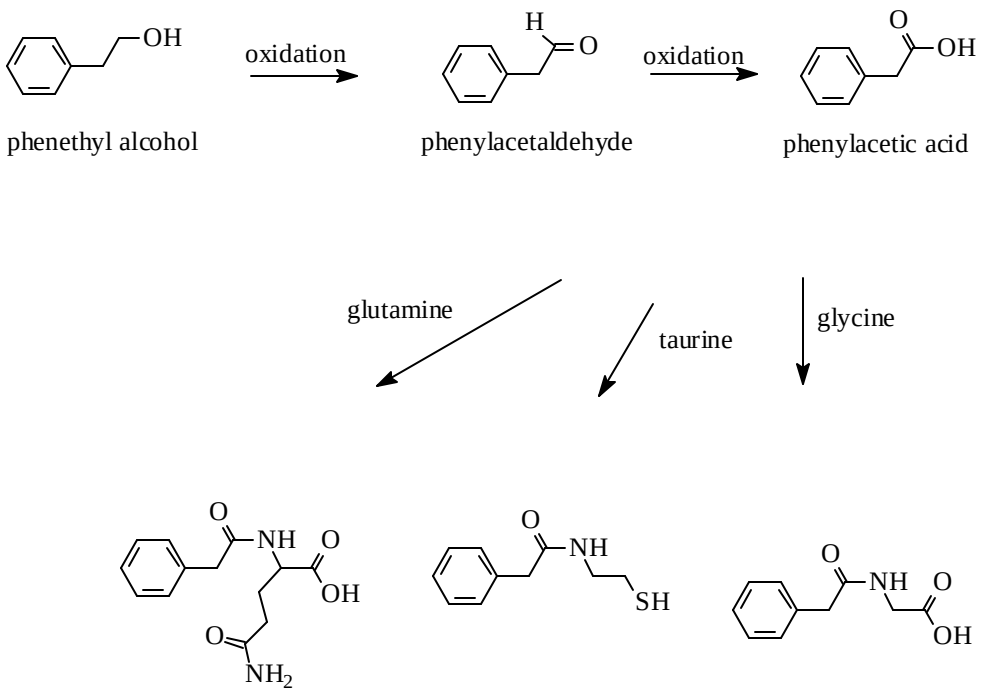
In humans, 26% of a 4,000 mg oral dose of phenethyl alcohol (No. 987) is excreted in urine as the glutamine conjugate of phenylacetic acid within 24 hours [Thierfelder and Schempp, 1917]. In rabbits, 42% and 5% of a single 300 mg/kg bw oral dose of phenethyl alcohol is excreted in the urine as glycine and glucuronic acid conjugates, respectively, of phenylacetic acid within 24 hours. The ether soluble acid extracted from the 24-hour urine accounted for 61% of the dose [Bray *et al.*, 1958]. In an earlier study, 77% of 1300 mg/kg bw dose of phenethyl alcohol administered to rabbits *via* gavage was isolated from the 24-hour urine as an ether soluble acid. No appreciable quantity (less than 0.5%) of free phenylacetic acid was recovered [Bray *et al.*, 1946]. In another study it was reported that only 0.4 – 3.1% of an oral dose of phenylacetic acid was excreted unconjugated in the urine of rabbits [Tulane and Lewis, 1933].

Greater than 98% of a single oral dose of 80 mg of [carboxy-^{14}C]-phenylacetic acid administered to each of three healthy human volunteers and three patients exhibiting phenylketonuria was excreted in the urine within 24 hours as the glutamine conjugate [James *et al.*, 1973]. Greater than 98% of a 1 mg/kg oral dose of [carboxy-^{14}C]-phenylacetic acid given to two male volunteers was excreted in the urine within 24 hours [James *et al.*, 1972]. Based upon the results of studies using radiolabelled phenylacetic acid, it may be concluded that phenylacetic acid is rapidly absorbed and excreted within 24 hours.

---

[2] V_{max} is the maximum rate or velocity of an enzymatic reaction which is indicative of all of the enzyme active site(s) is complexed with substrate.

FIGURE 1. METABOLISM OF PHENETHYL ALCOHOL



3 TEST PLAN

3.1 Chemical and Physical Properties

3.1.1 Melting Point

The measured melting point of phenethyl alcohol has been reported to be –27 °C [CRC, 1986; Merck, 1996]. Based on the input data of –27 °C, the calculated melting point of phenethyl alcohol is reported to be –6.0 ^{o}C (adapted Joback method) [MPBPVP EPI Suite, 2000a].

3.1.2 Boiling Point

The measured boiling point of phenethyl alcohol has been reported to be 218 ^{o}C [CRC, 1986] and 219 - 221 ^{o}C at 750 mm Hg [Merck, 1996]. Based on input values of 218.2 ^{o}C for boiling point and -27 ^{o}C for melting point, the calculated boiling point is 224.8 ^{o}C (adapted Stein and Brown Method) [MPBPVP EPI Suite, 2000a].

3.1.3 Vapor Pressure

Two measured values for vapor pressure of phenethyl alcohol are in good agreement. The vapor pressure has been reported to be 0.0868 mm Hg at 25 °C [MPBPVP EPI Suite, 2000b] and 0.0707 mm Hg at 30 °C [Vuilleumier, 1995]. Based on input values of 218.2 ^{o}C for boiling point and -27 ^{o}C for melting point, the calculated vapor pressure is 0.0222 mm Hg at 25 °C [MPBPVP EPI Suite, 2000a].

3.1.4 n-Octanol/Water Partition Coefficients

The reported log Kow of phenethyl alcohol is 1.36 [Sangster, 1989; KOWWIN EPI Suite, 2000b]. Log Kow was also calculated resulting in a value of 1.57 [KOWWIN EPI Suite, 2000a]. The agreement between measured and calculated values confirms the experimental value of log Kow for phenethyl alcohol of 1.36.

3.1.5 Water Solubility

The measured water solubility for phenethyl alcohol is 22,200 mg/L [WSKOWWIN EPI Suite, 2000b] and 20,340 mg/L [Merck, 1996]. Based on an experimental melting point of –27 ^{o}C and a log Kow of 1.36, the calculated water solubility is reported to be 3,272 mg/L at 25 ^{o}C [WSKOWIN EPI Suite, 2000a].

3.1.6 New Testing Required

None.

3.2 Environmental Fate and Pathways

3.2.1 Photodegradation

The calculated photodegradation half-life for phenethyl alcohol is 12.6 hours [AOPWIN EPI Suite, 2000]. The calculations are based on measured rate constants for radical reactions of OH, O_3 and NO_3 with organic substrates [AOPWIN EPI Suite, 2000]. The short half-life is consistent with the presence of reactive benzylic hydrogen and alcoholic OH function in phenethyl alcohol. Therefore, the half-life can be considered reliable.

3.2.2 Stability in Water

Phenethyl alcohol will not hydrolyze in water. The molecule is expected to be stable in water.

3.2.3 Biodegradation

Phenethyl alcohol has been subjected to a CO_2 production test according to OECD Guideline 301B [Quest International Ltd., 1994]. The total biodegradation was 106.3% after 28 days with 10% degradation in approximately 1 day. Phenethyl alcohol can be considered to be readily and ultimately biodegradable.

The calculated value of 103.0% linear biodegradation probability is in agreement with experimental values [BIOWIN EPI Suite, 2000].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1996a; 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, measured melting point (-27 °C), boiling point (218.2 °C), vapor pressure (0.089 mm Hg at 25 °C), water solubility (20,340 mg/L) and log Kow (1.36).

The model predicts that phenethyl alcohol is distributed mainly to the soil (52%) and water (42%) [Mackay, 1996a; 1996b].

In these environmental compartments, released phenethyl alcohol exhibits a potential to be oxidized to the corresponding carboxylic acid. Because of its use in food and cosmetics, soaps and detergents, the majority of phenethyl alcohol will enter the environment primarily *via* a sewage treatment plant and will be rapidly and extensively biodegraded.

3.2.5 New Testing Required

None. Phenethyl alcohol has been shown to be readily and ultimately biodegradable. While fugacity calculations estimate that the bulk will end up in soil and water, this does not take into account the principal uses of phenethyl alcohol, which would result in exposure *via* a sewage treatment plant allowing for rapid and extensive biodegradation.

3.3 Ecotoxicity

3.3.1 Acute Toxicity to Fish

Phenethyl alcohol has been subjected to a 96-hour static acute toxicity test according to the German guideline 38-414 with Golden Orfe *(Leuciscus idus*). An LC50 of between 220 mg (0 mortality) and 460 mg (100% mortality) was reported [BASF AG, 1988c]. The experimental value [ECOSAR EPI Suite, 2000] of LC50 of 230 mg/L is conservative since it approximates experimental LC0 value.

3.3.2 Acute Toxicity to Invertebrates

Phenethyl alcohol has been subjected to a 48-hour acute toxicity guideline study with *Daphnia magna*. A 48-hour EC50 of 287 mg/L was reported [BASF AG, 1988a]. The calculated [ECOSAR EPI Suite, 2000] LC50 of 239 mg/L is in the same range as the measured value.

3.3.3 Acute Toxicity to Aquatic Plants

Phenethyl alcohol has been subjected to a 72-hour growth inhibition test with algae (*Scenedesmus subspicatus*). The reported EC50 was 490 mg/L [BASF AG, 1988b]. The model value for the 96-hour EC50 is 146 mg/L [ECOSAR EPI Suite, 2000]. Although the model prediction is more conservative, it is on the same order of magnitude as the measured value.

3.3.4 New Testing Required

None. The acute aquatic toxicity of phenethyl alcohol has been well characterized in fish, invertebrates and plants and indicates a low order of toxicity.

3.4 Human Health Data

3.4.1 Acute Toxicity

Phenethyl alcohol has been subjected to acute oral, dermal, inhalation and intraperitoneal tests in rats, mice, rabbits, and guinea pigs. The rat oral LD50 values range from 1500 mg/kg bw to 2540 mg/kg bw [Jenner *et al*., 1964; Carpenter *et al*., 1974; Zaitsev and Rakhmanina, 1974; International Flavors & Fragrances, Inc., 1982; Moreno, 1982a].

The reported dermal LD50 values are in considerable disagreement ranging from 805 mg/kg [Carpenter *et al*., 1974] to 2535 mg/kg in the rabbit [International Flavors & Fragrances, Inc., 1983] to greater than 5000 mg/kg in the rat [Moreno, 1982b]. The intermediate value, 2535 mg/kg is from the best-documented study and is most consistent with what would be expected based on the dermal penetration in rabbits of 46-56% obtained from a pharmacokinetic study (Hawkins *et al*., 1987, no robust summary provided) and the oral LD50 values discussed above.

An acute inhalation exposure of phenethyl alcohol aerosol in rats for a 4-hour period followed by a 14-day observation resulted in no deaths and the LC50 was reported to be greater than 4.63 mg/L [Breckenridge *et al*., 1980].

Based on these data, it is concluded that phenethyl alcohol exhibits a very low acute toxicity.

3.4.2 Genetic Toxicity

3.4.2.1 In vitro Genotoxicity

No evidence of mutagenicity was observed when phenethyl alcohol [Florin *et al*., 1980] was incubated with *Salmonella typhimurium* (SAL) strains TA98, TA100, TA1535 and TA1537 with and without S-9 metabolic activation at concentrations up to and including 3 micromol/plate. No increase in a sister chromatid exchange was observed when human whole-blood lymphocyte cultures were exposed to 2-phenethyl alcohol for 72 hours

[Norppa and Vainio, 1983]. Also, no increase in unscheduled DNA synthesis was noted when rat hepatocytes were incubated with its principal metabolite phenylacetic acid [Heck *et al*., 1989].

3.4.2.2 In vivo Genotoxicity

In vivo mutagenicity and genotoxicity data exist for two structurally related substances that participate in the same metabolic pathway as phenethyl alcohol. One is a phenylacetic acid ester, isoeugenol phenylacetate and the other is 2-methyl substituted phenylacetaldehyde. Phenylacetic acid esters undergo hydrolysis prior to absorption. The methyl, ethyl, isopropyl, isoamyl, citronellyl esters of phenylacetic acid are rapidly hydrolyzed *in vitro* in simulated gastric juice and pancreatic juice [Longland *et al*., 1977] or in a buffered solution of pancreatin [Grundschober, 1977]. Once formed phenylacetic acid is excreted as the glutamine conjugate.

Given the rapid rate of formation of phenylacetaldehyde derivatives from the corresponding phenethyl alcohol derivatives *in vivo* [Bosron and Li, 1980; Pietruszko *et al*., 1973] and the rapid conversion of phenylacetaldehyde derivatives to phenylacetic acid metabolites [Martini and Murray, 1996], the structurally related aldehyde participates in the same metabolic pathway utilized by phenethyl alcohol.

None of the two structurally related substances (a phenethyl aldehyde and phenylacetic acid ester) showed any evidence of genotoxicity in well-recognized *in vivo* assays (mouse micronucleus and sex-linked recessive lethal assay). In mammals, substances were administered orally, by gavage, or by intraperitoneal injection at doses that were significant fractions of the reported lethal dose levels.

No increase in the frequency of sex-linked recessive mutations occurred in a three brood study when *Drosophila melanogaster* were maintained on 10 mM of phenylacetaldehyde, 2-methyl or 25 mM solutions of phenylacetic acid, isoeugenol ester for 3 days [Wild *et al.*, 1983].

In two clastogenicity assays, groups of 10- to 14-week-old NMRI mice were intraperitoneally injected at 0 and 24 hours with 564, 987, or 1,410 mg/kg bw of

phenylacetic acid, isoeugenol ester or at 0 hours with 134, 401, or 670 mg/kg bw of phenylacetaldehyde, 2-methyl [Wild *et al.*, 1983]. At 30 hours, the mice were sacrificed and bone marrow smears were prepared using the staining method of Schmid (1976). There was no evidence of micronucleated polychromatic erythrocytes for treated or control groups.

Based on the results of this *in vivo* genotoxicity assays for a structurally related phenethyl aldehyde and phenylacetate ester and the lack of any evidence of genotoxicity for numerous *in vitro* assays with and without metabolic activation for phenethyl alcohol, it is unlikely that phenethyl alcohol would exhibit a significant genotoxic potential *in vivo*. No additional *in vitro* and *in vivo* assays are requested for this substance.

Given that the *in vitro* and *in vivo* results consistently demonstrate that the substances exhibit a low order of genotoxic potential, no additional studies are required.

3.4.3 Repeated Dose Toxicity

A 90 day dermal toxicity study has been reported for phenethyl alcohol at daily doses of 250, 500, 1,000 or 2,000 mg/kg bw. The two highest dose groups exhibited a statistically significant lower growth rate than controls but with no significant differences in degree: final body weights (g) 1 g/kg males 482 ± 56, females 276 ± 16; 2 g/kg males 484 ± 43, females 272 ± 16. There was also a statistically significant decrease in hemoglobin and white blood cell count in males at the high dose. No significant effects on clinical examination, hematology, urinalysis or histopathological examination were seen. The no observable adverse effect level (NOAEL) was concluded to be 500 mg/kg bw/day [Owston, *et al*., 1981]. Based on the high dermal penetration of phenethyl alcohol on rats (70% after 5 daily repeated doses of 140 mg/kg bw; Hawkins *et al*., 1986, 1988, 1990), this translated to an internal dose of 350 mg/kg bw/day.

There are no acceptable oral repeated dose studies with phenethyl alcohol, however, the lack of serious effects in the dermal 90-day study combined with the high degree of dermal penetration make this an acceptable alternative. Furthermore, a 17-week study is available for a phenethyl ester that hydrolyzes to phenethyl alcohol and phenylacetic acid prior to absorption [Longland *et al*., 1977; Grundschober, 1977]. For 17 weeks, rats were

maintained on diets containing 1,000, 2,500 or 10,000 ppm of phenethyl phenylacetate. These dietary levels were calculated to provide an average daily intake of approximately 50, 125 or 500 mg/kg bw/day. No adverse effects were observed at any of the three dietary levels [Hagan *et al*., 1967]. While this study was conducted prior to GLP, it was conducted by the U.S. Food and Drug Administration and can be classified as highly reliable.

Additionally, a study of phenethyl alcohol in a mixture is available. Groups of male and female Wistar albino rats (20/sex/group) were given a mixture of compounds dissolved in tap water as their only drinking source for 56 weeks. This mixture included 6,000 mg/kg bw ethyl alcohol (6%), 4 mg/kg bw ethyl acetate (0.004%), 120 mg/kg bw isoamyl alcohol (0.12%), 120 mg/kg bw phenethyl alcohol (0.12%), 200 mg/kg bw isobutyl alcohol (0.2%), and 200 mg/kg bw acetic acid (0.2%)[3]. A control group of 20 rats/sex was maintained on tap water only. Body weights were recorded weekly. The activity of alcohol dehydrogenase (ADH), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and the protein content were determined at two-four week intervals in the livers of rats. At study termination, liver, kidney, heart, spleen, and lung were examined histologically. There was no difference in absolute or relative liver weight between the test and control groups. There was a slight increase in GOT activity between 28 and 56 weeks in both the test and control groups. Histopathological examination revealed no significant abnormalities in any of the organs examined. The authors concluded that the mixture of chemicals containing phenethyl alcohol did not produce any effects in the parameters tested [Johannsen and Purchase, 1969].

3.4.4 Reproductive Toxicity

A reproductive/developmental screening test has been performed for the principal metabolite phenylacetic acid. The lack of toxicity to reproductive organs in subchronic toxicity tests (see section 3.4.3), the lack of developmental toxicity in females in numerous developmental studies at high dose levels of phenethyl alcohol, indicate that phenethyl alcohol exhibits a low order of reproductive toxicity.

---

[3]Conversions of dose based on FDA, 1993.

Four groups of 10 virgin Crl CD rats were administered oral dose levels of 0, 250, 500, or 1,000 mg/kg bw of phenylacetic acid by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Vollmuth *et al.*, 1995]. Maternal indices monitored included twice-daily clinical observation, measurement of body weights, food consumption, duration of gestation, and fertility parameters (mating and fertility index, gestation index, number of offspring per litter). Offspring indices included daily observation, clinical signs, examination for gross external malformations, and measurement of body weight.

At 250, 500 and 1,000 mg/kg in dams, a significant (P less than 0.05) decrease in body weight and absolute and relative food consumption was reported during the premating period. Clinical signs of toxicity and a statistically significant increase in mortality was recorded in the mid- and high dose groups, but not in the low dose dams. Necropsy of dams showed gross lesions in the mid- and high-dose groups. Measurements of mating success and fertility were similar for controls, low-dose and mid-dose groups. No changes in fertility index, averages for duration of cohabitation or gestation, gestation index, implantation sites, litter size, or pup sex ratios were seen at any dose levels. The only reproductive parameter affected was a decrease in the number of females mated per number of females pregnant at the 1000 mg/kg bw level. Based on the toxicity and increased dam mortality at the two highest dose levels and a decrease in mating index in the mid-dose group, the maternal reproductive effects were reported at 500 and 1,000 mg/kg bw/day. The dose level of 250 mg/kg bw/day had no adverse effects on the reproductive performance of female Sprague-Dawley rats [Vollmuth *et al.*, 1995].

3.4.5 Developmental/Teratogenicity Toxicity

Screening studies performed by one group of investigators during the 1980's reported that low dose levels of phenethyl alcohol and phenylacetic acid produce teratogenic effects resembling Fetal Alcohol Syndrome [Mankes *et al*., 1983]. (Mankes, 1984 and 1985 were presentation abstracts and no robust summaries provided). These results are contradicted by the results of another study in which phenethyl alcohol given to pregnant rats at high doses at critical periods of embryogenesis do not cause any visible anomalies

in embryonal development [Bottomley *et al.*, 1987]. More recent comprehensive studies conducted with high dose levels of phenethyl alcohol given either by oral [Bottomley *et al.*, 1987] and dermal [Palmer *et al.*, 1986] routes of exposure have demonstrated that this group of substances exhibits a very low order of developmental toxicity.

In the original studies [Mankes *et al.*, 1983, 1984 and 1985], pregnant Long Evans rats were given oral doses of 4.3, 43 or 432 mg/kg of phenethyl alcohol by gavage during days 6 to 15 of gestation. The average birth weight and pup size of all treated groups were significantly lower than those of the control group, but the change was not dose-related. In fact, birth weights were greater in the mid-dose group than in controls. Mean litter size was greater in the high dose group (13) than in either the two lower doses (9) or controls (12). Also, embryolethality did not occur in the high dose group but was 18% at 43 mg/kg and 10% at 4.3 mg/kg. The authors reported a clear dose related increase in the percentage of malformations in live offspring (100% at the 432 mg/kg level, 93% at 43 mg/kg and 50% at 4.3 mg/kg). Malformations were mainly in ocular malformation, neural tube defects, hydronephrosis and limb defects [Mankes *et al.*, 1983]. In abstracts of subsequent studies reported by the same authors [Mankes *et al.*, 1984; 1985], dose levels of phenethyl alcohol equivalent to 0.02% and 24% of the oral LD50 were administered to pregnant Long Evans rats. Intrauterine growth retardation (birth weight reductions) and embryolethality were reported at all dose levels. These observations are inconsistent with those of the original study.

The effects of dietary administration of microencapsulated phenethyl alcohol on pregnancy of the rat was studied [Bottomley *et al.*, 1987] according to a protocol essentially the same as OECD 414. The test diet containing nominal 0 (control), 1,000, 3,000, or 10,000 ppm (approximately 0, 50, 150, or 500 mg/kg bw) was made available to the rats during days 6 to 15 of pregnancy. Spray-dried gum Arabic, the microencapsulant, was used as a placebo control and was also added to the lower concentrations so that the total inclusion level remained constant for all groups at 5%. The animals were killed on day 20 post coitum and *in utero* development assessed by determination of litter values and examination of the fetuses for structural malformations or anomalies. Achieved intake of phenethyl alcohol was calculated for dams during the treatment period, values were adjusted to take account of the assayed content of test

material in the microcapsules used and indicated that the actual intake was about 83, 266, and 799 mg/kg per day for groups designated 1,000, 3,000 and 10,000 ppm, respectively. The treatment of the dam with phenethyl alcohol by dietary inclusion of 799 mg/kg had a negligible detrimental effect on *in utero* development. Although there was clear evidence of impaired weight gain in dams following initial exposure to the test material, fetal development was virtually unaffected, the only possible exception being a marginal delay in the ossification process, an event that the authors indicated is usually transient and self-correcting during postnatal maturation. At 83 and 266 mg/kg, phenethyl alcohol did not elicit any overt response in the dam and embryofetal development and morphology was unaffected [Bottomley *et al.*, 1987].

The effect of phenethyl alcohol on pregnancy of rats was studied following a similar protocol to OECD 414. Phenethyl alcohol was applied topically at the dose of 0, 0.14, 0.43 or 1.40 ml/kg during day 6 to 15 of pregnancy. The doses are approximately equal to 0, 140, 430, and 1400 mg/kg bw, respectively, and were chosen so that the intermediate dose was roughly equivalent to the highest dosage used in a previous oral study [Mankes *et al*., 1983]. The highest dose was designed to extend the range in case of differential absorption by the dermal route. The animals were killed on day 20 of pregnancy and *in utero* development assessed by determination of litter values and examination of the fetuses for soft tissue and skeletal changes. At 1.40 ml/kg per day, there was clear evidence of both maternal toxicity including lethality, suppression of mean food intake and growth rate and embryo-fetal toxicity indicated by resorption, embryo-fetal wastage, reduction in mean litter size, depression of fetal weight, a wide range of soft tissue and skeletal changes, incomplete ossification. For the latter, the pattern of response and the comprehensive nature of the morphological changes were considered by the authors, to be beyond those that would occur merely as a secondary consequence of the maternal response. In this study, 0.43 ml/kg per day was considered close to the threshold of maternal toxicity but while there was no evidence of an adverse effect on litter values, there was a dose-dependent increase in some of the morphological changes recorded in fetuses. A dose of 0.14 ml/kg per day did not elicit any adverse effects in the litter values. Based on the overt effects on fetal development at the higher dosages, the slight differences in morphological changes between the 0.14 ml/kg dose and controls (cervical

rib(s) thoracic vertebral irregularities), the authors concluded that the 0.14 ml/kg dose level (140 mg/kg bw) is a threshold for developmental toxicity in the rat [Palmer *et al*., 1986].

In order to better clarify the fetal NOAEL in the previous study, a limited developmental study was conducted by a similar protocol, but looking particularly at the cervical rib bud and thoracic vertebrae effects, pregnant rats were treated dermally with 70, 140, 280, 430 or 700 mg/kg bw/day on days 6 to 16 of pregnancy. Cervical rib buds were statistically significantly higher than controls at 700 mg/kg only and there were no significant incidences of vertebrae effects. However, significant and dose-related skin irritation was seen in the dams at all dose groups and delayed ossification (judged to be reversible) was seen in fetuses of all groups. The only statistically significant difference from controls in the two lower dose groups was incomplete ossification of the pelvis but with no dose correlation. These effects may have been secondary to the dermal irritation. No clear no observable effect level (NOEL) for dams or fetuses can be concluded from this study, however, the minor effects seen in the two lower doses could lead to a conclusion of a fetal NOAEL of 140 mg/kg bw [Christian *et al*., 1988].

In the reproduction/developmental screening test discussed in the section on reproductive toxicity, four groups of 10 virgin Crl CD rats were administered oral dose levels of 0, 250, 500, or 1,000 mg/kg bw of phenylacetic acid by gavage once daily, 7 days prior to cohabitation, through cohabitation (maximum of 7 days), gestation, delivery, and a 4-day post-parturition period. The duration of the study was 39 days [Vollmuth *et al.*, 1995]. Offspring indices monitored included daily observation, clinical signs, examination for gross external malformations, and measurement of mortality (number of stillborns), viability (pups dying on days 1-4), body weight and body weight gain. The only effects reported occurred at the 1000 mg/kg bw/day level. A statistically significant decrease in viability and a non-significant decrease in body weight gain were reported at the highest dose level. The dose level of 500 mg/kg bw/day had no adverse effects on the development of the offspring of female Sprague-Dawley rats.

3.4.6 New Testing Required

None.

3.5 Test Plan Table

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 60-12-8 Phenethyl alcohol	**A, Calc**	**A, Calc**	**A, Calc**	**A, Calc**	**A, Calc**

Chemical	Environmental Fate and Pathways			
	Photodegra-dation	Stability in Water	Biodegra-dation	Fugacity
CAS No. 60-12-8 Phenethyl alcohol	**Calc**	**NA**	**A, Calc**	**Calc**

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 60-12-8 Phenethyl alcohol	**A, Calc**	**A, Calc**	**A, Calc**

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 60-12-8 Phenethyl alcohol	**A**	**A**	**R**	**A**	**R**	**A, R**

LEGEND

Symbol	Description
R	Endpoint requirement fulfilled using data for structurally related substances, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

4 REFERENCES FOR TEST PLAN AND ROBUST SUMMARIES

AOPWIN EPI Suite (2000) US Environmental Protection Agency.

BASF AG (1988a) Labor Oekologie, unpublished data (0107/88).

BASF AG (1988b) Labor Oekologie, unpublished data (1010/88).

BASF AG (1988c) Abteilung Toxikologie, unpublished data (87/410).

Bauer K. and D. Garbe (1985) Common Flavor and Fragrance Materials Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

BIOWIN EPI Suite (2000) US Environmental Protection Agency.

Block W. (1953) On the physiology of C[14]-radioactive mescaline in animals. Part IV. Comparative studies with C[14]-mescaline and C(14)-*beta*-phenylethylamine. *Zeitschrift fur Naturforschung,* **8B**, 440-444.

Bosron W.F. and Li T.K. (1980) Alcohol dehydrogenase. In Enzymatic Basis of Detoxication Edited by W.B. Jakoby vol. 1, pp. 231-248, Academic Press, Orlando FL.

Bottomley A. M., Ratcliffe H. E., John D. M., Anderson A., Dawe I. S. (1987) Effect of Dietary Administration of Micro-Encapsulated Phenylethyl Alcohol on Pregnancy of the Rat (Embryotoxicity Study). Unpublished report.

Bray H. G., Neale F. C. and Thorpe W. V. (1946) The Fate of Certain Organic Acids and Amines in the Rabbit. *Biochemical Journal,* **40**, 134-139.

Bray H.G., James S.P., and Thorpe W.V. (1958) Metabolism of some omega-halogenoalkylbenzenes and related alcohols in the rabbit. *Biochemical Journal,* **70**, 570-579.

Breckenridge C., Collins C.J., Qureshi S. and Procter B.G. (1980) The acute toxicity of inhaled phenyl ethyl alcohol in the albino rat. Unpublished report to RIFM.

Caldwell J. (1987) Human disposition of [14C]-ORP/178. Private communication to FEMA. Unpublished report.

Carpenter C.P., Weil, C.S., and Smyth, H.F. (1974) Range-finding toxicity data: List VIII. *Toxicololgy and Applied Pharmacology,* **28**, 313-319.

Christian M.S. and Hoberman A.M. (1988) Dosage-range developmental toxicity (embryo/fetal toxicity and teratogenicity) study of 2-phenylethylalcohol (PEA) administered dermally to presumed pregnant mice. Unpublished report to RIFM.

CRC Handbook of Chemistry and Physics (1986) 67th edition, Robert C. Weast, editor, The Chemical Rubber Co Press, Inc. Boca Raton, Florida.

El Masry A.M., Smith J.N. and Williams R.T. (1956) Studies in detoxication. 69. The metabolism of alkylbenzenes: n-propylbenzene and n-butylbenzene with further observations on ethylbenzene. *Biochemical Journal,* **64**, 50-57.

ECOSAR EPI Suite (2000) US Environmental Protection Agency, OPPT Risk Assessment Division (G. Cash & V. Nabholz, April 2001).

Fassett D.W. (1963) Toxicity of phenethyl alcohol. *Industrial Hygiene and Toxicology*, 1476-1477.

Florin I., Rutberg L., Curvall M. and Enzell C. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. *Toxicology*, **18**, 219-232.

Grundschober F. (1977) Toxicological assessment of flavouring esters. *Toxicology*. **8**, 387-390.

Hagan E. C., Hansen W. H., Fitzhugh O. G., Jenner P. M., Jones W. I., Taylor J. M., Long E. L., Nelson A. A. and Brouwer J. B. (1967) Food Flavourings and Compounds of related Structure. II. Subacute and Chronic Toxicity. *Food and Cosmetic Toxicology,* **5**, 141-157.

Hall R.L. (1960) Recent progress in the consideration of flavoring ingredients under the food additives amendment. *Food Technology,* **14**(10), 488-495.

Hawkins D.R., Elsom L.F., Girkin R., Bigs, S.R. and Williams S.G.F. (1988) The percutaneous absorption and disposition of (14)C-2-phenylethanol in rabbits. Unpublished report to RIFM.

Hawkins D.R., Elsom L.F., Girkin R., Huckstep M.and De-Salis C.M. (1987) The dermal absorption of (14)C-2-phenylethanol in man following a single topical application. Unpublished report to RIFM.

Hawkins D.R., Elsom L.F., Girkin R. and Jackson R. (1986) Dermal absorption and disposition of (14)C-2-phenylethanol in rats. Unpublished report to RIFM.

Hawkins D.R., Redrup M.J., Brindley C.J. and Williams S.G.P (1990) Plasma and urine concentrations and pharmacokinetics of phenylacetic acid and phenylethanol in the rat following single doses of phenylethanol administered *via* different routes. Unpublished report.

Hawkins D.R. and Mayo B.C. (1986) Plasma kinetics of [14C]-ORP/178 in the rat. Private communication to FEMA. Unpublished report.

Heck J. D., Vollmuth, T. A., Cifone, M. A., Jagannath, D. R., Myhr B., and R.D. Curren (1989) An evaluation of food flavoring ingredients in a genetic toxicity screening battery. *The Toxicologist*, **9(1),** 257.

International Flavors & Fragrances, Inc. (1982) Acute oral toxicity study of phenethyl alcohol in rats. Unpublished report.

International Flavors & Fragrances, Inc. (1983) Acute dermal toxicity test of phenethyl alcohol in rabbits. Unpublished report.

International Organization of the Flavour Industry (IOFI) (1995) European inquiry on volume use. Private communication to Flavour and Extract Manufacturers Association (FEMA).

James M.O., Smith R.L. and Robert L. (1973) Conjugation of phenylacetic acid in phenylketonurics. *European Journal of Clinical Pharmacology,* **5(4),** 243-246.

James M.O., Smith R.L., Williams R.T. and Reidenberg M. (1972) The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. *Proceedings Royal Society London*, B, **182(1066**), 25-35.

Jenner P. M., Hagan E. C., Taylor J. M., Cook E. L. and Fitzhugh O. G. (1964) Food Flavourings and Compounds of Related Structure. I. Acute Oral Toxicity. *Food and Cosmetics Toxicology,* **2**, 327-343.

JECFA Joint FAO/WHO Expert Committee on Food Additives (2002) Safety Evaluation of Phenethyl Alcohol, Aldehyde, Acid, and Related Acetals and Esters Used as Flavouring Agents (2002-2). In press.

Johannsen E. and Purchase I.F.H. (1969) Kaffircorn malting and brewing studies. XXI: The effect of the fusel oils of Bantu beer on rat liver. *S.A. Medical Journal (Supplement- S.A. Journal of Nutrition),* **43**(12), 326-328.

Kay H.D. and Raper H.S. (1922) Mode of oxidation of fatty acids with branched chains. II. The fate in the body of hydratropic, tropic, atrolactic and atropic acids together with phenylacetaldehyde. *Biochemistry Journal,* **16**, 465-474.

Klimisch H. J., Andreae, M., and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Journal of Regulatory Toxicology and Pharmacology*, **25**, 1-5.

KOWWIN EPI Suite (2000a) US Environmental Protection Agency.

KOWWIN EPI Suite (2000b) US Environmental Protection Agency (Hansch C. *et al*., 1995).

Klyosov A. A. (1996) Kinetics and Specificity of Human Liver Aldehyde Dehydrogenases Toward Aliphatic, Aromatic and Fused Polycyclic Aldehydes. *Biochemistry,* **35(14),** 4457-4467.

Longland R.C., Shilling W.H. and Gangolli S.D. (1977) The hydrolysis of flavouring esters by artificial gastrointestinal juices and rat tissue preparations. *Toxicology,* **8**, 197-204.

Lucas C.D., Putnam J.M., and Hallagan J.B. (1999) Flavour and Extract Manufacturers Association (FEMA) of the United States 1995 Poundage and Technical Effects Update Survey. Washington D.C. Self-published.

Maarse H., Visscher C.A., Willemsens L.C. and Boelens M.H. (2000) Volatile Components in Food-Qualitative and Quantitative Data. Centraal Instituut Voor Voedingsonderzioek TNO. Zeist, The Netherlands.

Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626.

Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Maganova N.B. and Zaitsev A.N. (1973) Study of the Embryotoxic Action of Some Synthetic Food Flavourings. *Vopr Pitan*, **32(4),** 50-54.

Mankes R. F., LeFevre R., Bates H. and Abraham R. (1983) Effects of Various Exposure Levels of 2-Phenylethanol on Fetal Development and Survival in Long-Evans Rats. *Journal of Toxicology and Environmental Health,* **12**, 235-244.

Mankes R. F., LeFevre R. and Abraham R. (1984) Reproductive Effects of Some Solvent Alcohols: Evaluation of Structure-Activity Relationships in 2-Substituted Ethanols. *Journal of the American College of Toxicology,* **3(2),** 166.

Mankes R. F., LeFevre R., Renak R., Fiesher J., and Abraham R. (1985) Reproductive effects of some solvent alcohols with differing partition coefficients. *Teratology*, **31(3),** 67A.

Martini R. and Murray M. (1996) Rat Hepatic Microsomal Aldehyde Dehydrogenase. Identification of 3- and 4-Substituted Aromatic Aldehydes as Substrates of the Enzyme. *Chemistry Research in Toxicology,* **9**, 268-276.

Merck Index (1996) 12th edition, Susan Budavari, editor, Merck & Co. Inc. Whitehouse Station, NJ.

Moreno O.M. (1982a) Acute toxicity studies in rats. Private communication to FEMA.

Moreno O.M. (1982b) Acute toxicity studies in rats. Private communication to FEMA.

MPBPVP EPI Suite (2000a) US Environmental Protection Agency.

MPBPVP EPI Suite (2000b) US Environmental Protection Agency (Daubert T.E. and Danner, R.P., 1989)

Norppa H. and Vainio H. (1983) Induction of Sister-Chromatid Exchanges by Styrene Analogues in Cultured Human Lymphocytes. *Mutation Research,* **116**, 379-387.

Opdyke D.L.J. (1975) Monographs on fragrance raw materials, *Fd. Comet. Toxicol*., **33,** (Supplement) 903.

Owston E., Lough R. and Opdyke D.L. (1981) A 90-day study of phenylethyl alcohol in the rat. *Fd and Cosmet Toxicol*, **19(6),** 713-715.

Palmer A.K., Bottomley, A. M., Ratcliffe, H.E. Clark, R., and John, D. M. (1986). Effect of Phenylethyl Alcohol (PEA) on Pregnancy of the Rat. Huntingdon Research Center. Unpublished report to RIFM.

Pietruszko R., Crawford K. and Lester D. (1973) Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver, and yeast towards saturated and 2-enoic alcohols ands aldehydes. *Archives of Biochemistry and Biophysics*, **159**, 50-60.

Quest International Ltd. (1994). The ultimate biodegradability of phenylethyl alcohol in the sealed vessel test. Unpublished report.

Richter D. (1938) Elimination of amines in man. *The Biochemical Journal*, **32**, 1763-1769.

Sandler M., Ruthven C.R.J., Goodwin B.L., Lees A. and Stern G.M. (1982) Phenylacetic acid in human body fluids: High correlation between plasma and cerebrospinal fluid concentration values. *J Neurol. Neurosurg. Pshychiat*., **45(4),** 366-368.

Sangster S.A. and Lindley M.G. (1986) Metabolism and excretion of ORP/178 in man. Private communication to FEMA. Unpublished report.

Sangster J. (1989) Octanol-water partition coefficients of simple organic compounds. *J Phys. Chem. Ref. Data,* **18(3),** 1111-1229.

Seakins J.W.T. (1971) The determination of urinary phenylacetylglutamine as phenylacetic acid: Studies on its origin in normal subjects and children with cystic fibrosis. *Clinica Chimica Acta.* **35(1),** 121-131.

Simpson V. J., Baker R. and Deitrich R. A. (1985) Inducible Aldehyde Dehydrogenases from Rat Liver Cytosol. *Toxicology and Applied Pharmacology*, **79**, 193-203.

Stofberg J. and Grundschober, F. (1987) Consumption ratio and food predominance of flavoring materials. *Perfumer and Flavorist*, **12**, 27.

Thierfelder H. and Schempp E. (1917) Behaviour of benzoylpropionic acid, phenethyl alcohol and phenoxyacetic acid in the body of men and dogs. *Arch. Ges. Physiol.* **167**, (280-288).

Tulane V. J. and Lewis H. B. (1933) Studies in the synthesis of hippuric acid in the animal organism. IX. A comparative study of the rate of synthesis and excretion of hippuric and phenaceturic acids by the rabbit. *Journal of Biological Chemistry*, **103**, 151-160.

Vollmuth T.A., Bennett, M.B., Hoberman, A.M. and Christian, M.S. (1995) An Evaluation of Food Flavoring Ingredients Using an In Vivo Reproductive and Developmental Toxicity Screening Test. *Teratology*, **41(5**), 597.

Vuilleumier C., Flament I., and Sauvegrain P. (1995) Headspace analysis study of evaporation rate of perfume ingredients applied to skin. *International Journal of Cosmetic Science*, **17**, 61-76.

Wild D., King, M.T., Gocke, E. and Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the salmonella/microsome, basc and micronucleus tests. *Fd Chem Toxicol*., **21(6),** 707-719.

Williams R.T. (1959) Detoxication mechanisms- The metabolism and detoxication of drugs, toxic substances and other organic compounds. 2nd ed. Chapman and Hall Ltd., London. pp. 796.

WSKOWIN EPI Suite (2000a) US Environmental Protection Agency.

WSKOWIN EPI Suite (2000b) US Environmental Protection Agency (Vivandi S.C., *et al*., 1981).

Zaitsev A. N. and Rakhmanina, N. L. (1974) Some Data on the Toxic Properties of Phenylethyl and Cinnamyl Alcohols. *Voprosy pitaniia*, **6**, 48-53.